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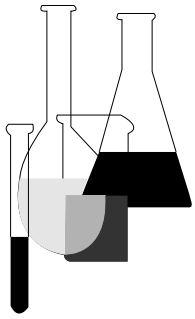
Health Effects Test Guidelines

OPPTS 870.5900
In Vitro Sister Chromatid Exchange Assay



“Public Draft”

INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Public Draft Access Information: This draft guideline is part of a series of related harmonized guidelines that need to be considered as a unit. *For copies:* These guidelines are available electronically from the EPA Public Access Gopher (gopher.epa.gov) under the heading ‘‘Environmental Test Methods and Guidelines’’ or in paper by contacting the OPP Public Docket at (703) 305–5805 or by e-mail: guidelines@epamail.epa.gov.

To Submit Comments: Interested persons are invited to submit comments. By mail: Public Docket and Freedom of Information Section, Office of Pesticide Programs, Field Operations Division (7506C), Environmental Protection Agency, 401 M St. SW., Washington, DC 20460. In person: bring to: Rm. 1132, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA. Comments may also be submitted electronically by sending electronic mail (e-mail) to: guidelines@epamail.epa.gov.

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OPPTS 870.5900 In vitro sister chromatid exchange assay.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source materials used in developing this harmonized OPPTS test guideline are OPPT 40 CFR 798.5900 In Vitro Sister Chromatid Exchange Assay and OECD 479 Genetic Toxicology: In Vitro Sister Chromatid Exchange Assay in Mammalian Cells.

(b) **Purpose.** The sister chromatid exchange (SCE) assay detects the ability of a chemical to enhance the exchange of DNA between two sister chromatids of a duplicating chromosome. The test may be performed in vitro, using, for example, rodent or human cells, or in vivo using mammals, for example, rodents such as mice, rats and hamsters.

(c) **Definitions.** The definitions in section 3 of TSCA and in 40 CFR Part 792—Good Laboratory Practice Standards (GLP) apply to this test guideline. The following definition also applies to this test guideline.

Sister chromatid exchanges are reciprocal interchanges of the two chromatid arms within a single chromosome. These exchanges are visualized during the metaphase portion of the cell cycle and presumably require enzymatic incision, translocation and ligation of at least two DNA helices.

(d) **Test method**—(1) **Principle.** Following exposure of cell cultures to test chemicals, they are allowed to replicate in the presence of bromodeoxyuridine (BrdU), followed by treatment with colchicine or colcemid to arrest cells in a metaphase-like stage of mitosis (*c*-metaphase). Cells are then harvested and chromosome preparations made. Preparations are stained and metaphase cells analyzed for SCEs.

(2) **Description.** In vitro SCE assays may employ monolayer or suspension cultures of established cell lines, cell strains, or primary cell cultures. Cell cultures are exposed to test chemical and are allowed to replicate in the presence of BrdU. Prior to harvest, cells are treated with a spindle inhibitor (e.g. Colchicine or Colcemid#) to accumulate cells in *c*-metaphase. Chromosome preparations from cells are made, stained and analyzed for SCEs.

(3) **Cells**—(i) **Type of cells used in the assay.** There are a variety of cell lines or primary cell cultures, including human cells, which may be used in the assay. Established cell lines and strains should be checked for *Mycoplasma* contamination and may be periodically checked for karyotype stability.

(ii) **Cell growth and maintenance.** Appropriate culture media and incubation conditions (culture vessels, temperature, humidity, and CO_2 concentration) should be used.

(4) **Metabolic activation.** Cells should be exposed to test chemical both in the presence and absence of an appropriate metabolic activation system.

(5) **Control groups—Concurrent controls.** Positive and negative (untreated and/or vehicle) controls, with and without metabolic activation, should be included in each experiment.

(6) **Test chemicals**—(i) **Vehicle.** Test substances may be prepared in culture media or dissolved or suspended in appropriate vehicles prior to treatment of the cells. Final concentration of the vehicle should not reduce cell viability or growth rate.

(ii) **Exposure concentrations.** Multiple concentrations of the test substance over a range adequate to define the response should be tested. Among the criteria to be taken into consideration for determining the upper limits of test chemical concentration are cytotoxicity and solubility. Cytotoxicity of the test substance may be altered in the presence of metabolic activation systems. Cytotoxicity may be evidenced by a large (e.g., 75 percent) decrease in the number of cells that have divided twice in the presence of BrdU. Relatively insoluble substances should be tested up to the limit of solubility. For freely soluble nontoxic chemicals, the upper test chemical concentration should be determined on a case by case basis. When appropriate, a positive response should be confirmed by using a narrow range of test concentrations.

(e) **Test performance**—(1) **Established cell lines and strains.** (i) Prior to use in the assay, cells should be generated from stock cultures, seeded in culture vessels at the appropriate density and incubated at 37 °C.

(ii) Cell lines and strains should be treated with test chemical both with and without metabolic activation when they are in the exponential stage of growth. At the end of the exposure period, cells should be washed and incubated for two replication cycles in medium containing BrdU. After BrdU is added, the cultures should be handled in darkness, under ‘‘safe’’ (e.g., darkroom) lights, or in dim light from incandescent lamps to minimize photolysis of BrdU containing DNA. At the end of the BrdU incubation period, cells should be fixed and stained for SCE determination. Cultures should be treated with a spindle inhibitor (e.g., colchicine or Colcemid®) 2 h prior to harvesting.

(2) **Human lymphocyte cultures.** (i) For preparation of human lymphocyte cell cultures, heparinized or acid-citrate-dextrose treated whole blood should be added to culture medium containing a mitogen, e.g.,

phytohemagglutinin (PHA) and incubated at 37 °C. White cells sedimented by gravity (buffy coat) or lymphocytes which have been purified on a density gradient such as Ficoll-Hypaque may also be utilized.

(ii) Cells should be exposed to the test chemical during at last two time intervals, e.g. G_0 and S. Exposure during the G_0 phase of the cell cycle should be accomplished by adding the test substance prior to addition of mitogen. Exposure during or after the first S phase may be accomplished by exposing cells 24–30 h after mitosis, under ''safe'' (e.g. darkroom) lights, or in dim light from incandescent lamps to minimize photolysis of BrdU containing DNA. At the end of the BrdU incubation period, cells should be fixed and stained for SCE determination. Cultures should be treated with a spindle inhibitor (e.g. colchicine or Colcemid®) 2 h prior to harvesting.

(3) **Human lymphocyte cultures.** (i) For preparation of human lymphocyte cell cultures, heparinized or acid-citrate-dextrose treated whole blood should be added to culture medium containing a mitogen, e.g., phytohemagglutinin (PHA) and incubated at 37 °C. White cells sedimented by gravity (buffy coat) or lymphocytes which have been purified on a density gradient such as Ficoll-Hypaque may also be utilized.

(ii) Cells should be exposed to the test chemical during at least two time intervals, e.g., G_0 and S. Exposure during the G_0 phase of the cell cycle should be accomplished by adding the test substance prior to addition of mitogen. Exposure during or after the first S phase may be accomplished by exposing cells 24–30 h after mitogen stimulation. After exposure, cells should be washed and then cultured in the absence of the chemical.

(4) **Culture harvest time.** A single harvest time, one that yields an optimal percentage of second division metaphases, is recommended. If there is reason to suspect that this is not a representative sampling time (which may occur for short-lived, cycle specific chemicals), then additional harvest times should be selected.

(5) **Staining method.** Staining of slides to reveal SCEs can be performed according to any of several protocols. However, the fluorescence plus Giemsa method is recommended.

(6) **Number of cultures.** At least two independent cultures should be used for each experimental point.

(7) **Analysis.** Slides should be coded before analysis. The number of cells to be analyzed should be based upon the spontaneous control frequency and defined sensitivity and the power of the test chosen before analysis. In human lymphocytes, only cells containing 46 centromeres should be analyzed. In established cell lines and strains, only metaphases

containing ±2 centromeres of the modal number should be analyzed. Uniform criteria for scoring SCEs should be used.

(f) **Data and report**—(1) **Treatment of results.** Data should be presented in tabular form, providing scores for both the number of SCEs for each metaphase and the number of SCEs per chromosome for each metaphase.

(2) **Statistical evaluation.** Data should be evaluated by appropriate statistical methods.

(3) **Interpretation of results.** (i) There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the number of sister chromatid exchanges. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of the test substance concentrations.

(ii) A test substance which produces neither a statistically significant dose-related increase in the number of sister chromatid exchanges nor a statistically significant and reproducible positive response at any one of the test points is considered not to induce rearrangements of segments of DNA in this system.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) **Test evaluation.** (i) Positive results in the in vitro SCE assay indicate that under the test conditions the test substance induces reciprocal chromatid interchanges in cultured mammalian somatic cells.

(ii) Negative results indicate that under the test conditions the test substance does not induce reciprocal chromatid interchanges in cultured mammalian somatic cells.

(5) **Test report.** In addition to the reporting recommendations as specified under 40 CFR part 792, subpart J, the following specific information should be reported:

(i) Cells used, density at time of treatment, number of cell cultures.

(ii) Methods used for maintenance of cell cultures including medium, temperature, and CO_2 concentration.

(iii) Test chemical vehicle, concentration and rationale for the selection of the concentrations of test chemical used in the assay, duration of treatment.

(iv) Details of both the protocol used preparation of the metabolic activation system and its use in the assay.

(v) Growth period in BrdU; identity of spindle inhibitor, its concentration and duration of treatment.

(vi) Time of cell harvest.

(vii) Positive and negative controls.

(viii) Method used to prepare slides for SCE determination.

(ix) Criteria for scoring SCEs.

(x) Details of the protocol used for growth and treatment of human cells if used in the assay.

(xi) Dose-response relationship, if applicable.

(g) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Latt, S.A. et al. Sister chromatid exchanges: a report of the U.S. EPA's Gene-Tox Program. *Mutation Research* 87:17–62 (1981).

(2) [Reserved]